In the Specification

Amend the specification to insert the underlined sequence identification numbers in the paragraphs identified below.

First Paragraph on Page 13:

- -Figure 1. Autoradiography of labeled DNA products after fractionation on a polyacrylamide/ urea gel. Panel A shows products of the "A" extension reaction on oligonucleotide primer 182 [SEQ ID NO: 1] directed by template oligonucleotides 180 or 181. [SEQ ID NO: 2]. Panel B shows products of the "B" termination reaction on oligonucleotide primer 182 annealed to template oligonucleotides 180 or 181. Panel C shows the same products as in panel B after purification on magnetic beads. Note: oligodeoxynucleotide 182 was used as supplied by Midland Certified Reagents with no further purification. The minor bands above and below the main band are presumably contaminants due to incomplete reactions or side reactions that occurred during the step-wise synthesis of the oligonucleotide. For a definition of the "A" extension reaction and the "B" termination reaction, see "A. GENERAL METHODS" in the Detailed Description of the Invention.- -

Second Paragraph on Page 13:

- -Figure 2. Detection of Sequence Polymorphisms in PCR Products. Target polymorphic DNA sequence showing amplification primers [SEQ ID NO: 9] and [SEQ ID NO: 10], detection primers [SEQ ID NO: 11] and [SEQ NO: 12] and molecular clone (plasmid) designations. For each primer, sites of binding to one or the other strand of the target DNA sequence [SEQ ID NO: 13] are indicated by underlining, and the direction of DNA synthesis is indicated by an arrow. Numbering for the target sequence is shown in the righthand margin. Polymorphic sites at positions 114 and 190 are indicated by bold lettering and a slash between the two polymorphic possibilities.- -

Paragraph on Bridging Pages 13 and 14

- -Figure 3. Autoradiogram of gel-analyzed polymorphism test on PCR products. Templates from PCR products of p183, p624, or p814 were analyzed with the detection primers, TGLI182 [SEQ ID NO: 11] and TGLI166 [SEQ ID NO: 12] in a template-directed chain extension experiment, as described in the specification. Reaction products were fractionated by size on a polyacrylamide/urea DNA sequencing gel, and incorporation of [^{35}S]-α-thio-dideoxy adenosine monophosphate was assayed by autoradiography.- -

First Full Paragraph on Page 14

- -Figure 4. Gel electrophorectic analysis of the labeled extension products of primers [SEQ ID NO: 4] and TGL391 [SEQ ID NO: 3]. Productive primer-template complexes of TGL346 or TGL391 with the bead-bound oligonucleotide template, TGL382 [SEQ ID NO: 5], were subjected to primer extension labeling reactions with the four different [α-thio-^{35}S] dideoxynucleoside triphosphate mixes. Labelled primer DNA was released from the washed beads and electrophoresed on an 8% polyacrylamide/8 M urea DNA sequencing gel (2.5 moles of primer/lane), then analyzed by autoradiography. The four lanes shown for the primer TGL346 indicate that labeling occurred predominantly with the ddC mix, indicating that the next unpaired based in the TGL382 template adjacent to the 3' end of TGL346 was a G (see sequence given in Example 4). The four lanes shown for the primer TGL391 indicate that the labelling occurred predominantly with the ddT mix, indicating that the next unpaired base in the TGL382 template adjacent to the 3' end of TGL391 was an A.- -

First Paragraph on Page 15

- -Figure 6. PCR-amplified polymorphic locus of mammalian DNA. Shown is a 327 basepair segment of mammalian DNA that was amplified from samples of genomic DNA using the PCR primers TGL240 [SEQ ID NO: 6] (biotinylated) and TGL239 (unbiotinylated). Samples of DNA from two homozygous individuals, ESB164 (genotype AA) and EA2014 (genotype BB) [SEQ ID NO: 15], were subjected to the analyses described in Example 5. The complete DNA sequence [SEQ ID NO: 14] of the A allele at this locus is shown, with the polymorphic sites where the B allele sequence differs from the A allele sequence indicated by the bases underneath the A sequence. The detection primer, TGL308 [SEQ ID NO: 8], is shown base-paired with the template strand extending from the biotinylated primer. For the A allele, the first unpaired template base immediately downstream of the 3' end of TGL308 is a C, and for the B allele this base is an A. Thus, the A allele should result in labelling of TGL308 by the ddG mix only, and the B allele should result in labeling by the ddT mix only.- -

Second Paragraph on Page 15

- -Figure 7. Gel electrophoretic analysis of PCR products from two different homozygous individuals. Primers TGL240 [SEQ ID NO: 6] and TGL239 [SEQ ID NO: 7] were used to amplify genomic DNA (obtained from blood) from two individuals, ESB164 and EA2014. The products of the extension reactions for primer TGL308 [SEQ ID NO: 8], annealed to the bead-bound, PCR-generated template as outlined in Figure 7, were analyzed by electrophoresis on an 8% polyacrylamide/8 M urea DNA sequencing gel as outlined in Figure 5. Shown for individual ESB164 (genotype AA: labeling expected from the ddG mix) are 250 fmoles of extended primer from the four different ddNTP labelling reactions. Shown for individual EA2014 (genotype BB: labelling expected from the ddT mix) are loadings of 25, 75, and 250 fmoles of extended primer from the four different ddNTP labeling reactions.- -

First Paragraph on Page 16

--Figure 8. Autoradiographic analyses of total and NaOH-eluted radioactivity from TGL308 [SEQ ID NO: 8] primer extension reactions. Primer TGL308 was used to analyze the genotypes of individuals ESB164 and EA2014 as outlined in Example 5 and Figures 7 and 8. Total bead-associated radioactivity was determined by directly spotting a suspension of beads containing 75 fmoles of primer onto filter paper followed by autoradiographic detection of the label in the spot. Radioactivity specifically associated with the TGL308 primer was determined by magnetically immobilizing the beads, eluting the primer with NaOH as described in Examples 4 and 5, and spotting on filter paper an amount corresponding to 75 fmoles. Label in these spots was also detected by autoradiography. - -

First Paragraph Following Heading "Example 1" on Page 35

- -Primer oligo 182: $^{5'}$GCCTTGGCGTTGTAGAA$^{3'}$ [SEQ ID NO: 1]

Template oligos

180(C)/191(T): $^{3'}$TCGGGTCGGAACCGCAACATCTT^{C} /TATAGACTA$^{5'}$

[SEQ ID NO: 2]- -

Paragraph Bridging Pages 36 and 37

- -Example 2

- -The experiment described in Example 1 shows template-directed labeling of oligonucleotide primer 182 [SEQ ID NO: 1] in which the labeling is specific with respect to oligonucleotides or other species that migrate similarly on a polyacrylamide gel. In order to assess more generally the template-directed specific labeling of oligonucleotide 182 with respect to all other labeled species, regardless of gel mobility, a direct measurement of incorporated radioactivity was performed. In this experiment, both reactions "A" and "B" were performed, reaction products were purified using Dynabeads, and total radioactivity in the aliquots was measured by liquid scintillation counting. This procedure assesses both misincorporation of label into other species and, in addition, the efficiency of the Dynabead washing procedure with respect to

unincorporated nucleotides. As a practical matter, it would be of interest to minimize both sources of non-specific label in order to have a simple, non-gel-based, procedure for assessing specific, template-directed labeling of the primer. The results of directly counting the reaction products after washing on the magnetic beads are as follows (all results expressed as cpm of ^{35}S):- -

First Paragraph on Page 38

--Two amplification primers, TGL 105 [SEQ ID NO: 9] and TGL 106 [SEQ ID NO: 10] (Figure 2), were used to amplify a cloned stretch of bovine DNA containing two DNA sequence polymorphisms: a C or T at position 114 and an A and G at position 190 [SEQ ID NO: 13] (Figure 2). DNAs containing those polymorphisms were molecularly cloned and available on plasmids, as follows: plasmid p183, C114 and A190; plasmid p624, T114 and A190; plasmid p814, C114 and G190. Four PCR reactions with biotinylated primers were performed to amplify and purify specific strands of these plasmids for use as templates:

- Primers	Plasmids	Detection Primers
- 105 biotinylated	p183 and p624	TGL 182 [SEQ ID NO: 11]
- 106 unbiotinylated		
- 105 unbiotinylated,	p183 and p814	TGL 166 [SEQ ID NO: 12]
- 106 biotinylated		

The duplex PCR products were bound to magnetic microspheres, denatured with NaOH, and biotinylated strand purified as described above. Templates prepared with biotinylated TGL 105 were subjected to analysis by DNA sequencing with unbiotinylated primer TGL 106 in order to measure the amount of template present. Similarly, template prepared using biotinylated TGL 106 was analyzed by sequencing with unbiotinylated TGL 105.- -

Paragraph Bridging Pages 38 and 39

- -Approximately equal amounts of template (2 pmoles) were annealed for 5 min at 65°C to the polymorphism detection primers, TGL 182 [SEQ ID NO: 11] and TGL 166 [SEQ ID NO: 12] (see above and Figure 2). These primers hydrogen-bond to the templates in a sequence-specific fashion such that their 3'-termini are adjacent to nucleotide positions 114 and 190, respectively (Figure 2) [SEQ ID NO: 13]. Template-directed primer extension reactions (reaction "B" conditions) were carried out on these primer:template complexes in the presence of the four ddNTPs, one of which (ddATP) was labeled. The products of these extension reactions were analyzed by electrophoresis on a 15% polyacrylamide/8M urea gel followed by autoradiography (Figure 3).- -

First Paragraph Following Heading "Example 4" on Page 39

- -Primer oligo TGL391: $^{5'}$TGTTTTGCACAAAAGCA$^{3'}$ [SEQ ID NO: 3]
Primer oligo TGL346: $^{5'}$GTTTTGCACAAAAGCAT$^{3'}$ [SEQ ID NO: 4]
Template oligo TGL382: $^{3'}$CACAAAACGTGTTTTCGTAGGA$^{5'}$- biotin: (streptavidin-bead) [SEQ ID NO: 5] - -

First Paragraph bridging 41 and 42

- -TGL240: $^{5'}$AGATGATGCTTTTGTGCAAAACAC$^{3'}$ [SEQ ID NO: 6]
TGL239: $^{5'}$TCAATACCTGAGTCCCGACACCCTG$^{3'}$ [SEQ ID NO: 7]
TGL308: $^{5'}$AGCCTCAGACCGCGTGGTGCCTGGT$^{3'}$ [SEQ ID NO: 8]

Olignonucleotide TGL240 [SEQ ID NO: 6] was synthesized with a primary amino group attached to its 5' terminus and coupled with biotin as described above. TGL240 (biotinylated) and TGL239 (unbiotinylated) were used to amplify, via the polymerase chain reaction procedure (see "A. General Methods"), a region of DNA comprising a particular genetic locus in samples

of mammalian genomic DNA. DNAs from two different individuals, each homozygous for a particular set of linked sequence polymorphisms (the "A" allele and the "B" allele – see Figure 6), were examined. After the PCR reaction, 2-20 pmoles of duplex PCR DNA was incubated with 100 µl of streptavidin-conjugated M-280 Dynabeads (7 x 10^8 beads/ml) in TNET buffer in order to bind the biotinylated strand to the beads. After binding, the beads were magnetically immobilized and washed three times with 200 µl of TNET, then resuspended in 100 µl of TNET. To remove the non-biotinylated strand, 500 µl of 0.15 N NaOH was added and the suspension incubated for 30 minutes at 20°C. The beads were then magnetically immobilized and washed once with 250 µl of 0.15 N NaOH, three times with 500 µl TNET, and resuspended in 100 µl of TNET.- -

Paragraph Bridging Pages 42 and 43

- -The detection primer, oligonucleotide TGL308 [SEQ ID NO: 8] (Figure 6), was annealed to the bead-bound PCR-generated template as described above in Example 4. Further washes, extension reactions, and detection assays were also carried out as described in Example 4. A gel autoradiographic analysis of the labelled primer extension products for the two homozygous individuals, ESB164 ("AA" genotype) [SEQ ID NO: 14] and EA2014 ("BB genotype) [SEQ ID NO: 15], is shown in Figure 7. Autoradiographic analyses of total bead-bound radioactivity, or primer-associated radioactivity after NaOH elution, are shown for these same individuals using the filter spotting assay (Figure 8). For the analysis of primer only, 10 µl of 0.4 N NaOH was added to 10 µl of the bead suspension. After incubation for 10 minutes at room temperature, the beads were immobilized magnetically and the supernatant withdrawn and spotted onto nylon blotting membrane.- -